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Mesenchymal stem cells-based drug delivery systems for diabetic foot ulcer: A review

Mesenchymal stem cells for diabetic foot ulcer

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Abstract

Abstract: The complication of diabetes, which is known as diabetic foot ulcer (DFU), is a significant concern due to its association with high rates of disability and mortality. It not only severely affects patients' quality of life, but also imposes a substantial burden on the healthcare system. In spite of efforts made in clinical practice, treating DFU remains a challenging task. While mesenchymal stem cell (MSC) therapy has been extensively studied in treating DFU, the current efficacy of DFU healing using this method is still inadequate. However, in recent years, several MSCs-based drug delivery systems have emerged, which have shown to increase the efficacy of MSC therapy, especially in treating DFU. This review summarized the application of diverse MSCs-based drug delivery systems in treating DFU and suggested potential prospects for the future research.

Key Words: Diabetic foot ulcer; Mesenchymal stem cells; Drug delivery systems; Diabetes; Wound healing

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Core Tip: Diabetic foot ulcer (DFU) is a significant concern due to its association with high rates of disability and mortality. Mesenchymal stem cell (MSC) therapy has been extensively studied in treating DFU, the current efficacy of DFU healing using this method is still inadequate. However, in recent years, several MSCs-based drug delivery systems have emerged, which have shown to increase the efficacy of MSC therapy, especially in treating DFU.

INTRODUCTION

Diabetes, along with its associated metabolic complications, has emerged as one of the most rapidly growing global health emergencies in the 21st century[1-3]. Diabetic foot

ulcer (DFU) is a multifaceted and troubling complication of diabetes that poses significant challenges to healthcare providers and patients. DFU is characterized by the development of chronic wounds on the feet, which can lead to severe morbidity and mortality rates if left untreated[4-6]. Approximately 19-34% of diabetics experience DFU, a condition with a recurrence rate of 40% within one year. This poses a significant threat to patients' physical and mental well-being and places a substantial financial strain on both patients and their families[7]. The 10th edition of the International Diabetes Federation (IDF) Diabetes Atlas has revealed a sobering reality concerning the global burden of diabetes. With an estimated 536.6 million diabeticcases diagnosedworldwide in 2021, diabetes has become a significant public health crisis that demands immediate attention and action. Even more alarming is the prediction that this number will surge to a staggering 783 million by 2045 if current trends continue. Furthermore, it is anticipated that the healthcare costs associated with diabetes and its complications could globallyreach a staggering 966 billion USD in 2021[8-10]. The reported statistics revealed that DFU, a condition that affects the skin tissue of the feet and can cause serious complications, has a prevalence of 6.3% among the global population. It is noteworthy that this condition is more frequently observed in men than in women, indicating a gender-based disparity in its incidence rate. Moreover, the data suggested that patients with type 2 diabetes (T2D), a chronic metabolic disorder, are at a higher risk of developing DFU, with an estimated prevalence rate of 6.4%, as opposed to those with type 1 diabetes, who have a lower rate of approximately 5.5%. The prevalence of DFU varies significantly across different regions of the world, as per available data. Specifically, North America has the highest recorded incidence rate of 13%, followed by Africa at 7.2%. Meanwhile, relatively lower rates of 5.1% and 5.5%were reported in Europe and Asia, respectively. The prevalence of DFU in Oceania is the lowest among the regions mentioned above, with a rate of 3.0%[11-13]. Previous studies have also demonstrated that DFU increases the risk of death by 2.5-fold in diabetics[14-15].

The synthesis of iodine (I)-polyvinyl alcohol (PVA)@polydopamine (PDA) microspheres was documented in Yang et al.'s study[16]. The aim was to attain computed

tomography images, drug loading and controlled release capabilities, as well as improved embolization of the liver portal vein. The *in vivo* embolization findings demonstrated the presence of focal necrosis in hepatocytes, along with necrotic cell fragments and infiltration of inflammatory cells in liver tissue. These observations provided evidence that the I-PVA@PDA microspheres exhibit a more potent embolization effect compared to PVA particles. Additionally, the I-PVA@PDA microspheres were utilized for the delivery and controlled release of 5-fluorouracil, a chemotherapeutic drug. The results showed an initial rapid release (29.74% released) within the first 24 h, followed by sustained release (34.48%) over a period of 72 h. In Ouyang et al.'s research[17], a multifunctional bio-hemostatic hydrogel (CODM) was prepared based on hydrogen bonding and Schiff base bonding by using modified alginate, polyvinylpyrrolidone (PVP), and carboxymethyl chitosan. The amino group-modified montmorillonite was uniformly dispersed in the hydrogel through amido bond formation with the carboxyl groups of carboxymethyl chitosan and oxidized alginate. The catechol group (-CHO) and PVP were able to form hydrogen bonds with the tissue surface, resulting in firm tissue adhesion and wound hemostasis. The addition of montmorillonite-NH2 further improved the hemostatic ability, surpassing that of commercial hemostatic materials. Furthermore, the photothermal conversion capability (derived from polydopamine) was synergized with the phenolic hydroxyl group, quinone group, and protonated amino group, effectively eliminating bacteria both *in vitro* and *in vivo*. In a recent review[18], it was reported that a multifunctional CH hyaluronic acid three-dimensional hydrogel possesses a notable capacity for water absorption. This property holds potential for its application in managing inflammatory bowel diseases (IBD), with a concentration on various aspects, such as adhesion, synergistic therapy, pH sensitivity, particle size, and temperature sensitivity. A desirable polymer hydrogel for hemostasis is expected to possess the following characteristics[18]: (1) it should exhibit a rapid gelation rate to promptly stop bleeding and promote active wound healing; (2) in dynamic and humid environments, the hemostatic hydrogel should demonstrate adequate adhesion and exceptional mechanical properties

to effectively seal the wound and prevent the displacement of the hemostatic hydrogel from the bleeding site; and (3) it should exhibit favorable biocompatibility. Furthermore, it is important for the hydrogel to exhibit controllable swelling behavior as overly swollen hydrogels may exert pressure on the surrounding tissue.

The physiological mechanism underlying wound healing encompasses a series of coordinated events, primarily involving inflammation, angiogenesis, and extracellular matrix (ECM) remodeling. However, DFU is associated withan abnormal microenvironment with prolonged inflammatory cell infiltration and slow angiogenesis and ECM remodeling, leading to impaired wound repair via the vascular and neurotrophic pathways. This in turn impedes local tissue regeneration and greatly reduces wound healing[19-22]. In addition, due to decreased granulocyte functions and chemotaxis, DFU cases are prone to infections[23-25]. The challenge of healing wounds in DFU is a multifaceted issue that arises from a combination of different factors. These factors include peripheral arterial disorders, which can impair blood flow and delivery of essential nutrients to the wound site, peripheral neuropathy, which can affect nerve function and lead to reduced sensation and poor healing response, foot deformities that can create pressure points and limit mobility, and also bacterial infections that can further complicate the healing process[26-29]. Wound debridement, which isthe standard treatment for DFUandinvolves surgical removal of thickened, necrotic, damaged or infected tissues, has been widely used in clinical practice;the wound isthen covered with dressing and/or treated with antibiotics to prevent infection[30-32]. A wound dressing that meets the ideal standards should facilitate a moist wound environment, shield against secondary infections, eliminate wound exudates, regulate biofilm formation, and stimulate tissue regeneration[33-34]. However, none of the existing dressings are able to meet all of these requirements, and due to the influence of various factors, traditional treatments can no longer achieve satisfactory outcomes[35-36]. DFU patients continue to face significant clinical hurdles when it comes to wound healing. Despite numerous chronic wound management techniques and treatments having been developed, including gene therapy, growth factor therapy, stem cell therapy, and

biomaterial application, successfully repairing these wounds remains a formidable task[37-38]. Because of their diverse characteristics that involve producing numerous growth factors, cytokines, and chemokines, as well as regulating immune responses, supporting the development of new blood vessels, and restructuring tissue, mesenchymal stem cells (MSCs) have demonstrated significant therapeutic capabilities in improving wound healing for cases with DFU[39-41]. Most of the current cell-based therapies are administered viasystemic or subcutaneous injection of cells[42-43]. However, MSCs cannot be delivered to the wound via the systemic route, as cells are mainly retained in the lung or liver[44-45]. Although intradermal injection of MSCs into the wound was reported to significantly improve healing[46-48], despite the promising potential of MSC therapy, the effectiveness of this approach is still hampered by challenges, such as inadequate cell localization and compromised cell viability at the injurysite[49]. To eliminate these problems, researchers have utilized delivery systemsto deliverstem cells to the site of injury, and these delivery systems have been suggested to significantly improve stem cell viability and wound implantation rates. Furthermore, the delivery system scaffold also provides a three-dimensional (3D) structure for stem cell migration, proliferation, and differentiation[50].Figure 1 illustrates the summary of the main content of this review.

In the context of MSCs-based treatment of DFU, we reviewed the advancements made in preclinical and clinical application of various delivery systems, as depicted in Figure 2.

Potential mechanism of action of MSC therapy for DFU

Stem cells exhibited diverse developmental potentials, enabling them to be classified into three categories: totipotent stem cells (comprising embryonic stem cells), pluripotent stem cells, and specialized stem cells (e.g., hematopoietic stem cells and neural stem cells). Furthermore, stem cells can be distinguished from embryonic and somatic stem cells based on their developmental stage. Embryonic stem cells are derived from embryonic and fetal tissues, while somatic stem cells are extracted from the organs or tissues of postnatal individuals. The two types of stem cells each have

advantages. However, the utilization of embryonic stem cells is subject to restrictions for several reasons, including the ethical controversy surrounding their use for medical purposes and the presence of legal constraints limiting their application. In addition, the sources of embryonic stem cells are limited. *In vitro* technologies for amplification and purification are still in an early stage of development. The utilization of allogeneic transplantation of embryonic stem cells in individuals of different genetic backgrounds is accompanied by the potential hazards of immune rejection and teratoma formation. In contrast, somatic stem cells, including hematopoietic stem cells, neural stem cells, liver stem cells, and MSCs, possess relatively lower immunogenicity and reduced risk of tumorigenesis, rendering them more appropriate for various clinical applications. The choice of stem cell type for a particular therapeutic intervention should be based on several important factors, including their safety profiles, efficacy, and compatibility with the recipient's immune system[51]. Clinical and animal experiments have indicated that therearetwo striking biological features of somatic stem cells. First, once transplanted, somatic stem cells undergo chemotaxis and arerecruited to the site of damage in massive numbers. Second, once they reach the site ofdamage, somatic stem cells undergo induced differentiationinto cells essential for damaged tissue repairin the local microenvironment. Site-specific differentiation is one of the mechanisms by which somatic stem cells promote damaged tissue repair, and researchers have revealed that MSCs promote reconstruction of the local microcirculation by releasing cytokines and growth factors through paracrine and endocrine effects;thisis the main mechanism by which these cells accelerate wound healing[52,53]. These findings may expand the indications for clinical treatment using somatic stem cells and facilitate the development of somatic cell and tissue engineering approaches. MSCs are nonhematopoietic stem cells resulting from mesoderm differentiation. They are adherent cells *in vitro* and can be massively expanded and differentiated into mesenchymal cells, also known as mesenchymal progenitor cells (MPCs). The latter can be further differentiated into various connective tissue cells, including adipose cells, osteocytes, chondrocytes, vascular endothelial cells, osteoblasts, myoblasts, and nerve cells[54]. MSCs are a versatile

cell population that can be obtained from various sources, including bone marrow, umbilical cord and blood, peripheral blood, fat, liver, gingiva, oral mucosa, amniotic fluid, as well as interstitial and connective tissues of organs. Due to the abundance of MSCs in these sources and their ability to differentiate into multiple cell types, they are valuable tools in regenerative medicine and tissue engineering research. One of the major advantages of MSCs is that their isolation does not pose ethical issues, unlike some other stem cells. Furthermore, studies have demonstrated that treatment with MSCs is safe and can lead to few side effects, providing another level of confidence in their use. The versatility and safety of MSCs make them an ideal candidate for utilization in tissue engineering investigations and clinical trials[55]. MSCs are easily isolated from different sources and have high proliferative potential and genetic stability. Theymigrate to damaged tissues, where they exhibit resistance to inflammation, influence the microenvironment, promote angiogenesis, and exert antifibrotic and antiapoptotic effects. Additionally, they release cytokines involved in damage repair and tissue regeneration, contributing to the healing process[56].

The intricate process of wound healing can be divided into four distinct overlapping phases, including homeostasis, inflammation, proliferation, and maturation. During the homeostasis stage, blood vessels in the affected area quickly constrict to minimize bleeding, while platelets form a plug to seal the injured site. During this stage, coagulation factors are activated, coordinating their efforts to form a fibrin clot that plays a vital role in stabilizing the wound. The inflammation phase is characterized by the influx of immune cells, such as neutrophils and macrophages, into the wound area. These cells are responsible for the removal of debris, pathogens, and damaged tissue. Additionally, they release growth factors and cytokines that initiate the subsequent phases of the healing process. In the proliferation stage, fibroblasts and endothelial cells start to proliferate and migrate into the wound bed, leading to the formation of new blood vessels and ECM. This stage also involves the deposition of collagen fibers, which provide structural support to the healing tissue. Finally, in the maturation phase, the newly formed tissue undergoes remodeling and maturation,

resulting in a stronger and more organized scar. This process can take several months to complete, during which time the collagen fibers realign and cross-link to increase the tensile strength of the tissue[57]. MSCs exerttherapeutic effects onDFU via several mechanisms. First, a key cause offailure of DFUhealing is poor blood supply to the site of the ulcer and disrupted angiogenesis. MSCs, through their ability to secrete various growth factors, have been shown to enhance angiogenesis,referring to the process of forming new blood vessels from pre-existing ones. This effect is mediated via both autocrine and endocrine signaling pathways, leading to the upregulation of several growth factors that are crucial for promoting angiogenesis. Vascular endothelial growth factor (VEGF) is a critical growth factor that stands out among others because of its ability to stimulate the proliferation and differentiation of endothelial cells, leading to the formation of new blood vessels. Basic fibroblast growth factor (bFGF), placental growth factor (PIGF), insulin-like growth factor 1 (IGF-1), and angiopoietin-1 (Ang-1) are also key players in promoting angiogenesis by inducing endothelial cell migration and proliferation. In addition to these growth factors, MSCs can also secrete stromal cell-derived factor-1 (SDF-1), erythropoietin (EPO), inducible nitric oxide synthase (iNOS), epidermal growth factor (EGF), and keratinocyte growth factor 2 (KGF-2). These growth factors work synergistically to further enhance angiogenesis and promote wound healing. The ability of MSCs to increase the levels of these growth factors is particularly relevant in DFUs wherein impaired angiogenesis is a significant contributing factor to poor wound healing. By improving blood flow in the affected area, MSCs can significantly accelerate the repair of DFUs, thus offering a promising therapeutic option for this debilitating condition[58]. Second, MSCs are involved in immunoregulation via different pathways, and they can improve the microenvironment, reduce the inflammatory response and alleviate tissueinjury[59]. (1) MSCs exert immunomodulatory effects by inhibiting T-cell activation[60]. T cells usually secrete a variety of proinflammatory factors after skin damage, delaying wound healing. MSCs secrete cytokines, including interferon-γ (IFN-γ), TNF-α, IL-1α or IL-1β, and nitric oxide synthase (NOS), which inhibit T-cell activation. In addition, MSCs can

block antigen-presenting cell (APC) maturation, thereby inhibiting the ability ofT cells to respond and exert immunomodulatory effects. (2) MSCs inhibit proinflammatory T cells, and immunomodulatory effects are mainly mediatedby Th17 cells and Treg cells[61]. One study showed that after the injection of bone-derived mesenchymal stem cells (BMSCs) into a mouse model of experimental allergic encephalomyelitis, Th17 cells were inhibited, accompanied by increases in the percentages of CD4+CD25+Foxp3+ Treg cells and IL-10-producing cells[62]. According to another report, MSCs modulate cytokine secretion by different T-cell subsets. Specifically, in experimental studies, it has been observed that the administration of MSCs results in a noticeable decrease in the secretion of certain proinflammatory cytokines, specifically FN-γ and TNF-α. On the other hand, there is a concomitant increase in the secretion of anti-inflammatory cytokines, such as IL-4 and IL-10. Moreover, the percentage of Treg cells was reported to increase after MSC treatment[63]. (3) MSCs exert immunomodulatory effects by reducing the number of classically activated macrophages (M1-type, proinflammatory) and increasing the number of selectively activated macrophages (M2-type, anti-inflammatory)[64]. It has been shown that the coculture of MSCs and macrophages reduces the overall numberof macrophages/monocytes, including M1 macrophages, but increases the percentage of M2 macrophages.In addition, MSCs induce M2 polarization of macrophages to exert immunomodulatory effects, enhancing wound repair. (4) MSCs exert immunomodulatory effects by reducing reactive oxygen species (ROS) levels[65]. In damaged tissues, macrophages engulf bacteria, apoptotic inflammatory cells or cell fragments, thereby killing pathogens and eliminating other harmful factors. However, the prolonged presence of neutrophils after phagocytosis usually results in massive production of ROS, which ultimatelycauses a respiratory burst and tissue injury. MSCs prevent excessive or improper activation of oxidative metabolism inneutrophils, while preserving the phagocytic ability of neutrophils. MSCs also inhibit neutrophil apoptosis, reducing ROS generation and the intensity of the respiratory burst. In summary, MSCs have exhibited to exert immunomodulatory effects, leading to the alleviation of inflammatory responses and tissue injury, as well as

the promotion of wound healing. Another important characteristic of MSCs is their ability to self-replicate and differentiate into different types of mature cells that possess distinct morphology, specific molecular markers, and specialized functions. This multidirectional differentiation potential allows for the generation of a diverse range of cell types, which has significant implications for regenerative medicine and other therapeutic applications involving tissue repair and regeneration[66]. MSCs can be divided into endothelial cells and keratinocytes that are involved in injury repair. Following transplantation of MSCs, there is a notable rise in the levels of angiogenic factors such as IGF-1, EGF, and IL-8. Moreover, the expression of keratinocyte-specific proteins and cytokeratin in wounds leads to the significant proliferation of various cell types, including epithelial cells and keratinocytes. These proteins expressed in wounds facilitate angiogenesis, epithelial cell regeneration, and wound healing. According to another study, in a rat model of DFU, MSCs were specifically localized to target ulcers, where keratin 19 secretion, formation of keratinocytes and ECM, and epithelial cell regeneration were promoted[67]. MSCs show promise for the treatment of DFU, and some encouraging results have been obtained from clinical trials. Further optimizationisneeded in terms of the following aspects oftreatment with MSCs: the feasibility of treatment using autologous and allogeneic MSC transplantation in patients with DFU,factors related totransplantation efficiency, the standardization of MSC quality detection methods and assessment criteria, MSC delivery systems, and methods to determine the survival rate of transplanted MSCs and the effectiveness and long-term efficacy of MSC transplantation. MSC therapy haspotential for promoting tissue regeneration and healing in DFU through the differentiation of MSCs into various cell types and the release of growth factors and cytokines. Furthermore, MSC therapy can enhance angiogenesis and blood vessel formation, increasing blood flow to the ulcerated area and promoting healing, while also preventing infections and reducing the need for antibiotics through its antimicrobial effects. Overall, these mechanisms suggest that MSC therapy may be a promising approach for treating DFU, and MSCs can provide neuroprotection by promoting nerve regeneration and reducing

neuropathic pain associated with DFU. MSCs possess the capacity to produce and release neurotrophic factors, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), which are potent mediators of nerve growth and survival. The secretion of these factors by MSCs can contribute to the repair and regeneration of damaged nerve tissues by promoting neuronal cell proliferation and differentiation, enhancing axonal sprouting and myelination, and reducing neuronal apoptosis.

Roles of MSCs-derived exosomes in DFUhealing

Medical professionals have been perplexed by the limited efficacy of MSCs in promoting wound healing. Low survival and proliferation rates of MSCs due to microenvironmental factors, such as ischemia, hypoxia, and inflammation, further affect the efficacy of MSCs-based treatment. An increasing body of research suggests that the transplantation of MSCs facilitates wound healing through two distinct mechanisms. One way is through direct differentiation, where MSCs differentiate into specific cell types such as fibroblasts, myofibroblasts, and endothelial cells, all of which contribute to tissue repair and angiogenesis. The other mechanism involves a paracrine effect, in which MSCs release various bioactive molecules, such as growth factors and cytokines, promoting the proliferation and migration of nearby cells involved in wound healing[68]. MSCs-derived exosomes act as mediators that deliver membrane receptors, proteins, mRNAs and microRNAs to receptor cells. Due to modulatory effects, gene expression and protein translation undergo changes in receptor cells, thereby influencing the biological activity of target cells. Exosome-based treatment provides a promising approach to overcome various limitations associated with stem cell-based treatment. These limitations include the challenge of large cell volume impeding capillary flow, the low dose and potency of stem cells, the potential presence of mutations or damaged DNA in stem cells, their potential to impair immunocompetence and the immune response, as well as their tendency to exhibit poor differentiation. Exosomes are paracrine products of stem cells and exert similar effects as stem cells. Exosomes are involved in a series of important processes during wound healing, including inflammatory regulation, angiogenesis, epithelial regeneration, and collagen deposition.

Bone marrow is the most common site forharvesting MSCs. The utilization of BMSCs that exert their therapeutic effects through paracrine exosomes has been the subject of extensive investigation in the treatment of DFU. For instance, Wang et al.[69] conducted a comprehensive analysis of the effects of exosomes originating from BMSCs on the tube-forming capabilities of endothelial progenitor cells (EPCs). Their results highlighted that BMSCs-derived exosomes exerted a significant modulatory influence on Nrf2, which ultimately led to reduced wound inflammation. Consequently, the exosomes played a vital role in promoting wound healing, re-epithelialization, collagen deposition, and angiogenesis in diabetic rats. Ding et al.[70] carried out subcutaneous injection of exosomes into skin wounds on the backs of diabetic rats.Following the transplantation of exosomes, the wound healing rate was notably higher in the group after 7 and 14 days versus that in the control group. The findings suggest that exosomes derived from BMSCs triggered the PI3K/AKT signaling pathway via miRNA-126-mediated PTEN downregulation, leading to proangiogenic characteristics both *in vivo* and *in vitro*. Furthermore, adipose-derived stem cells (ADSCs) also facilitate the repair of diabetic ulcers. ADSC-derived exosome transplantation may be a new method for treating DFU. A group of researchers conducted a study in which they extracted exosomes from ADSC-conditioned medium and combined them with EPC cells. Their findings indicated that the exosomes derived from ADSCs had a regulatory impact on LINC00511. This, in turn, impeded Twist1 ubiquitination and degradation induced by PAQR3. Ultimately, this process encouraged the proliferation, migration, and angiogenesis of EPCs, thereby accelerating the healing of DFUs[71]. Li et al.[72]revealed that ADSC-derived exosomes inhibited ROS and inflammatory cytokine production, thereby inhibiting EPC aging induced by high glucose and an oxidative microenvironment. Other effects included enhancing EPC viability, proliferation, and angiogenesis capacity and improving vascularization. Moreover, it was suggested that Nrf2-overexpressing ADSC-derived exosomes facilitated diabetic wound healing by enhancing collagen deposition, tissue fibrosis, and micro-angiogenesis. The mechanism may involveapromoting effect on vascularization and growth factor release and mitigation

of the oxidative stress response. In addition, researchers have demonstratedthe influences of human umbilical cord MSCs-derived exosomes on DFU healing. For instance, Hu et al.'s research involved investigating the effects of exosomes derived from human umbilical cord MSCs on promoting angiogenesis and fibroblast functions, leading to improved skin wound healing. This was achieved through PTEN inhibition by miR-21-3p and SPRY1, which resulted in enhanced healing of skin wounds[73]. Liu et al.[74] conducted a study to determine the efficacy of MSCs derived from human umbilical cords in improving wound healing and angiogenesis in a rat model of deep second-degree burn wounds. They observed a higher rate of wound closure and increased expression of CD31 *in vivo*. Furthermore, they found evidence suggesting that these MSCs facilitated the proliferation, migration, and tube formation abilities of human umbilical vein endothelial cells via exosome-mediated secretion of Ang-2. Despite these findings, there is a dearth of studies comparing the therapeutic potential of exosomes derived from MSCs of varied tissues in treating DFU, highlighting the need for further research in this area. There are still many problems to be resolved, such as the source and type of cells, isolation technique, dosage, transplantation method, and amplification methodforMSCs-derived exosomes. Preclinical and clinical studies with large sample sizes are still needed in the future.Exosomes derived from MSCs have emerged as critical players in the process of wound healing acceleration and promotion for individuals afflicted with DFU. These minute vesicles are laden with a plethora of bioactive molecules, including growth factors, cytokines, and microRNAs, that intricately regulate multiple cellular processes crucial to the wound healing cascade. Their multifaceted mechanisms of action make them an attractive therapeutic avenue for DFUs, as they not only promote angiogenesis and cell proliferation, but also modulate inflammation and ECM remodeling. Overall, MSCs-derived exosomes offer a promising approach to treat DFU, as they provide a safe and effective alternative to the use of whole cells. They can be easily obtained from MSCs through noninvasive techniques, and their administration has minimal risks compared with the use of whole cells (Figure 3).

MSC delivery viahydrogel scaffolds for DFU treatment

Hydrogels are complex structures made up of a mesh-like network of polymer chains that are chemically linked together. This unique arrangement allows hydrogels to absorb vast amounts of water, up to hundreds of times their own weight, while still maintaining their structural integrity. The implantation of hydrogels has been demonstrated to enhance surface cytocompatibility, antibacterial properties, and the preservation of cell viability at the targeted site, reflecting their capability in alleviatingwound healing[76-77]. Naturally derived hydrogels are biocompatible and biodegradable, interact with innate immune cells, and they are structurally similar to natural human tissues[78-80]. However, there has been a growing interest in the development of hydrogels with tailored properties and performance. However, studies have demonstrated that natural hydrogels possess a limited set of mechanical characteristics and tend to have significant fluctuations in properties between batches[81-82]. This limitation has led to a shift towards the use of synthetic hydrogels, which offer numerous advantages over their natural counterparts. Synthetic hydrogels have significantly longer lifetimes, higher water absorption capacities, and greater gel strengths compared to natural hydrogels[83-84]. Furthermore, they are known to be stable even under severe temperature fluctuations, making them ideal for a wide range of applications[85-86]. To capitalize on the unique properties of both types of hydrogels, composite hydrogels that combine natural and synthetic components have emerged as a promising alternative. These composite hydrogels offer the possibility of creating materials with controllable and customizable structures and functions, thereby expanding the range of potential applications[87-88]. Composite hydrogels are emerging as promising materials for tissue engineering due to their ability to be engineered with specific properties such as size, shape, surface activity, biodegradability, and biocompatibility. By carefully tailoring these characteristics, hydrogel scaffolds can provide a precise mechanical and biological environment to support cell growth and tissue regeneration[89]. New research has revealed that the use of type I rat tail collagen hydrogel to deliver murine BMSCs and adipose-derived MSCs (ADMSCs) could

noticeably modulate immune and inflammatory responses at wound sites. This could be attained via upregulating the expression levels of growth factors, including VEGF, as well as by attracting macrophages, which play a crucial role in tissue repair. This approach has been particularly successful in promoting wound healing in diabetic mice, where impaired immune function and chronic inflammation mainly hinder the natural healing process[90-93]. It has been demonstrated that collagen hydrogels can successfully deliver MSCs to the wound site and improve healing. Murine ADMSCs delivered via ahyperbranched polyethylene glycol diacrylate (PEGDA)-cross-linked gelatin hydrogel exhibit superior cell adhesion and are viable and metabolically active for 3 weeks[94-96]. Db/db diabetic mice that are injected with an ADMSC-loaded hydrogel at the wound surface have a significantly improved cell retention rate inthe wound, accelerated wound closure, enhanced angiogenesis, and attenuated inflammation[97]. In an effort to promote effective wound healing in the context of diabetes-induced impaired healing, innovative methods for delivering beneficial cells and substances have been investigated. Specifically, studies have revealed that the utilization of a biodegradable n-isopropylacrylamide thermo-sensitive hydrogel to deliver mouse BMSCs could remarkably improve wound healing in db/db mice. This delivery method has been demonstrated to enhance ECM deposition, angiogenesis, re-epithelialization, and granulation tissue formation within wounds. Additionally, it has exhibited the ability to regulate polarization of M1 and M2 macrophages at the wound site[98-99]. Additionally, the utilization of Pluronic F-127, a synthetic biocompatible hydrogel with unique thermo-sensitivity, offers an effective means of encapsulating and delivering numerous rat ADMSCs to the wound site. Such delivery has been found to stimulate angiogenesis and cell proliferation, ultimately leading to expedited wound healing in diabetic rats. These findings suggest the potential for novel therapeutic approaches utilizing these delivery methods to improve healing outcomes in individuals with diabetes-induced impairment[100-101]. Moreover, umbilical MSC implantation withPF-127 and sodium phosphate promotes wound healing and angiogenesis as well as improves dermal regeneration and collagen deposition in diabetic rats[102]. Diabetic rats that received

ADMSCs encapsulated in silk fibroin/chitosan hydrogel exhibited significantly increased re-epithelialization, granulosa tissue formation and capillary formation at the wound site 7 days after treatment[103]. Furthermore, the expression levels ofepithermal growth factor (EGF), TGF-β, and VEGF were also upregulated in the wound tissues on day 14 post-treatment. Utilizing hydrogels composed of hyaluronic acid and N-carboxyethyl chitosan cross-linked by adipic acid dihydrazide as a delivery mechanism, rat BMSCs have been found to effectively inhibit chronic inflammation, promote granulosa tissue formation, collagen deposition, nucleated cell proliferation, and stimulate angiogenesis in diabetic rats. As a result, these hydrogels can significantly enhance wound healing outcomes in this population[104]. Efforts to improve wound healing outcomes in individuals with diabetes-induced impairment have spurred innovative research in the field of cell delivery mechanisms. Researchers have discovered that delivering rabbit BMSCs via a nitric oxide-releasing S-nitroso-N-acetyl-penicillamine-loaded chitosan/polyvinyl-alcohol hydrogel can significantly enhance wound healing rates, re-epithelialization, and collagen deposition in diabetic rabbits, as described in literature sources[105-107]. Jin et al. developed an injectable hydrogel with unique properties such as suitable electrical conductivity and sustained hypoxia that can upregulate HIF-1α and connexin-43 expression in loaded ADMSCs, ultimately facilitating wound closure in diabetic rats. This hydrogel has been found to enhance angiogenesis, promoting the reconstruction of blood vessels, hair follicles, and dermal collagen matrix, further contributing to improved wound healing outcomes[108]. Srifa et al. conducted a study wherein they administered VEGFA-overexpressing human BMSCs to wounds in db/db mice either through direct injection or embedding them within a HyStem HP hydrogel. The researchers discovered that both methods of cell delivery enhanced the rate of wound healing; however, between days 7-9 after treatment, the hydrogel group exhibited significantly better wound healing compared to the direct injection group[109]. In a phase II clinical trial of MSC delivery viaa hydrogen scaffold (NTC02619877), the authors developed an allogeneic ADMSC hydrogel sheet that can maintain long-term stability under cryopreservation and has been approved for

marketing by the Ministry of Food and Drug Safety of South Korea (Approval No. ALL-ASC-DFU-201). This trial showed that 82% of diabetic patients had complete wound closure at week 12 after receiving the allogeneic ADMSC hydrogel sheet compared with 53% of controls, and adverse reactions were not observed after treatment, demonstrating that ADMSC delivery via thehydrogel is effective and safe for diabetic wound healing[110]. An in-depth case study was undertaken to explore the implications of utilizing sodium alginate hydrogel-encapsulated placenta-derived MSCs as a topical treatment for foot ulcers in patients with T2DM. The findings were exceptionally promising, with complete wound healing observed three weeks post-treatment, along with marked improvements in foot pain and minimal toxicity. Furthermore, no recurrence was noted during the six-month follow-up period[111]. However, as it wasa case study, further investigation is required.

At present, MSC delivery systems supported by hydrogelsmeet the need forlocal controlled release and create a 3D bionic environment. Hydrogel scaffolds not only increase bioavailability and antibacterial capacity by improvingtransport dynamics, but also provide a moist and stable wound repair environment for damaged ulcers, promoting synergy between skin cells and cytokines. Functional materials should be optimized in terms of the degree of crosslinking, porosity, swelling property, mechanical performance, cell adhesion, permeability, toxicity and cost efficiency. They should be synthesized with optimal healing strength to best mimic the ECM microenvironment to maintain the features and activity of each component. The development of hydrogel materials as MSC delivery systems or scaffolds holds significant potential for the future advancements in this field. In recent years, increasing research on the complex dynamics of biological systems has improved hydrogel material properties and hydrogel preparation methods and led to continual optimization of the interactions between organisms and scaffold materials. It can be concluded that the use of hydrogel scaffolds for the delivery of MSCs can significantly improve the therapeutic potential of MSCs-based treatments for DFU. Hydrogels offer a three-dimensional framework that maintains the viability, proliferation, and

specialization of cells, and simultaneously enables regulated discharge of growth factors that stimulate recovery of wounds and regeneration of tissues. Additionally, the mechanical properties of hydrogels can be modified to match the specific needs of DFU treatment, such as flexibility to accommodate weight-bearing or stiffness to support tissue regeneration. Overall, MSC-hydrogel therapies can effectively reduce inflammation, stimulate angiogenesis, and improve wound closure rates in human/animal models of DFU (Figure 4). However, there is an expectation for the development and application of a wider range of functional hydrogels that can perform distinct functions to facilitate DFU repair.

MSC delivery viafiber scaffolds for DFU treatment

Fiber scaffolds are 3D structures primarily composed of micro- or nanoscale fibers prepared by electrospinning to simulate the structure of natural human tissues[113]. The utilization of fiber scaffolds has been observed in multiple domains of tissue engineering, such as bone, cartilage, skin, vascular, and neural tissue engineering[114-116]. The notable surface-to-volume ratio of fiber scaffolds provides an ideal setting for cell adhesion, although the limited pore size may pose a challenge to cell migration. Consequently, the properties of fiber scaffolds should be tailored based on the specific cell type being cultured[117-118]. The employment of fiber scaffolds for the purpose of wound healing has recently garnered significant attention, as they have demonstrated a remarkable potential in promoting cell–cell and cell-ECM interactions, while also directing the functions and behaviors (e.g., cell morphology, proliferation, and differentiation) of diverse cells, including MSCs[119-122]. In the realm of diabetes wound healing, the utilization of fiber scaffolds for MSC transportation has been extensively employed. Chen and colleagues have devised a three-dimensional scaffold that is comprised of vertically or radially aligned nanofibers that can be customized to fit the size, depth, and configuration of different T2D wounds. The scaffold itself possesses an impressive ability to regain its shape both in water and the atmosphere, even after undergoing compression. When infused with BMSCs, this 3D fiber scaffold has the potential to stimulate the development of granulosa tissue, encourage angiogenesis, and

facilitate collagen deposition within the T2D wound[123-124]. Furthermore, Hou et al. developed a novel electrospun nanofibrous scaffold, which was composed of 80% polylactic acid, 10% silk, and 10% collagen. The scaffold was designed to deliver HO-1-overexpressing human BMSCs to wounds in diabetic mice. This hybrid scaffold has exhibited promising results in improving wound healing in diabetic mice. The authors found that this approachsignificantly improved angiogenesis and wound healing via the Akt signaling pathway[125]. He et al. delivered human BMSCs that overexpressed neurotrophic factors to wounds in diabetic miceviaan electrospun biomaterial, and this method was found to significantly accelerate wound closure and increase angiogenesis[126]. In addition, delivery of human ADMSCs to woundsviasilk fibroin scaffolds led to faster complete wound closure in db/db mice (d10) than in control mice (d15-17)[127-129]. It is easy to manipulate fiber scaffolds, but their preparation is verycomplicated. The dimensions and morphology of fiber scaffolds are affected by many factors, including solution viscosity, voltage, temperature, humidity, the distance between receiver and nozzle, and the loading flow rate of the solution. Ideal nanoscale fiber scaffolds can be fabricated only by systematically optimizing the above parameters. Fiber scaffolds offer a favorable setting for the growth and differentiation of MSCs, facilitate the regulated release of growth factors, and assist in the process of wound healing. Thus, MSC delivery viafiber scaffolds can improve wound closure rates, boost angiogenesis, reduce inflammation, and potentially offer better outcomes than other platforms for MSC delivery. Additional research is essential to enhance the design and production of fiber scaffolds for delivering MSCs, develop universally recognized procedures, and assess the enduring safety and efficacy of this technique.

MSC delivery viasponge scaffolds for DFU treatment

Scaffold sponges, which are widely used in tissue engineering and regenerative medicine, can be fabricated using a diverse range of techniques, involving porogen leaching, gas foaming, and freeze-drying. These methods enable the creation of scaffolds made from natural or synthetic polymers that possess a high degree of

porosity and a uniform network of interconnected pores[130-132]. Despite the longstanding use of sponge scaffolds in the biomedical field, researchers have long been striving to generate an environment that can provide support for the ECM in autologous cells and tissues. A 3D system with the ability to modulate cell viability and customize the structural and architectural properties, such as porosity, pore size, and interconnected dimension offers a significant degree of freedom. These features synergistically contribute to the regulation of cell-material interactions and consequently promote tissue growth within the scaffold gap[133-134]. Sponge and hydrogel scaffolds mainly differ in their method of fabrication, which results in differences in the water content of the scaffold. In contrast to hydrogels, the creation of sponge scaffolds is a time-consuming process that necessitates surface and structural modifications based on the type of cell and host tissue being used, as stated in the original citation. However, sponge scaffolds offer several potential benefits for skin wound healing. First, their highly porous structure closely resembles that of the ECM, which aids in supporting cell migration to the site of injury[135-137]. Second, because of their water absorption and retention capabilities, sponge scaffolds can absorb exudates from the wound site, providing a favorable environment for cell proliferation and migration[138-140]. The utilization of MSC scaffolds for diabetic wound healing frequently involves the use of sponge scaffolds made with collagen and chitosan. To create a collagen sponge scaffold, O'Loughlin et al. utilized freeze-drying techniques. Delivery of allogeneic BMSCs via topical application of the collagen sponge scaffold resulted in superior wound closure and angiogenesis on day 7 following implantation in diabetic rabbits when compared to the no treatment control group[141-142]. Tong et al. developed a collagen-chitosan sponge scaffold that is suitable for BMSC delivery by employing cross-linking and freeze-drying techniques, as mentioned in the original citation. This sponge scaffold has a 100 μm pore network and appropriate biodegradability and swelling ratio[143]. This type of scaffold creates an environment that is favorable for cell growth and stimulates hypoxia-pretreated rat BMSCs to produce higher levels of VEGF and platelet-derived growth factor (PDGF), as well as upregulate expression of key transcription factors, including HIF-1α, while

retaining cell viability. In STZ-induced diabetic rats, the BMSC-sponge scaffold group exhibited significantly improved wound closure, increased angiogenesis, and reduced inflammation (upregulated IL-10 gene and protein expression on days 7 and 14 post-implantation) versus the control group. Furthermore, Ni Annaidh et al. fabricated a sponge scaffold made of collagen and chitosan that was infused with simvastatin. The scaffold had high porosity, with pore sizes ranging from 20-200 μm, and possessed sufficient mechanical strength while maintaining elasticity similar to human skin. Additionally, the release of simvastatin from the scaffold could be controlled[144-146]. It was previously shown that delivery of rat ADMSCs by a sponge scaffold made of glycol chitosan and polyurethane combined with acupuncture had a synergistic immunomodulatory effect on wounds in mice with STZ-induced diabetes. This combination therapy improved wound closure and promoted complete re-epithelialization within 8 days,in contrastwith ADMSCs alone[147-151]. In addition, on day 8 after treatment, the wound displayed an increase in secretion of SDF-1 and TGFβ-1, while production of TNF-α and IL-1β was reduced. Additionally, sponge scaffolds have the potential to serve as a cell delivery system in conjunction with growth factors. Delivery of Balb/c mouse BMSCs by chitosan-alginate sponge scaffolds combined with EGF can enhance cell viability and transcription factor expression, maintain MSC pluripotency and self-renewal capability, and promote collagen deposition and angiogenesis by increasing granulosa tissue formation in the wounds of diabetic rats[152-155]. De Francesco et al. conducted a study to assess how effective autologous dermal micro-grafts, similar to MSCs, could be in treating DFUs by delivering them through collagen sponge scaffolds. The dermal micro-grafts were obtained through mechanical dissociation of small pieces of skin tissues and express MSC markers (e.g., CD34, CD73, CD90, and CD105) *in vitro*. The results showed that the micro-grafts remained viable and proliferative in the collagen scaffold, indicating that MSC-loaded sponge scaffolds could remarkably improve ulcer wound closure and enhance patients' quality of life[156-158].

A pore size of a few hundred microns is usually considered most suitable to ensure that cells obtain the needed nutrients. In addition, the porosity is generally above 70%, which provides enough space for cell penetration and mass transfer. Initial cell attachment is also guaranteed by the adequacy of the materials. The size of interconnections is a crucial factor that affects the transport characteristics of the entire porous structure. There seems to be a consensus that the minimal interconnection size is approximately 50 μm to allow angiogenesis and cell migration. The regulation of cell differentiation and function can be influenced by physical parameters, involving material hardness, viscoelasticity, and pore curvature. However, it is important to acknowledge the significant role that endogenous factors play in promoting complete cell maturation.Sponge scaffolds have demonstrated their effectiveness as delivery vehicles for MSCs, providing a supportive environment for cell growth and promoting tissue regeneration at the site of the ulcer. The utilization of sponge scaffolds has the potential to regulate the immune response and alleviate inflammation at the site of injury, thereby facilitating the process of wound healing. MSCs delivered by sponge scaffolds can improve angiogenesis and blood vessel formation, increasing blood flow to the ulcerated area and promoting healing. The controlled release of MSCs from sponge scaffolds can provide sustained therapeutic effects over time, reducing the need for frequent treatments. Compared to other methods of MSC delivery,the use of sponge scaffolds may improve patient compliance and reduce the risk of infection. The potential of MSCs delivered via sponge scaffolds to promote DFU wound healing is notable. These natural polymer-based scaffolds provide a 3D environment that supports MSC survival, proliferation, and differentiation, facilitating interactions with surrounding tissues. Overall, compared with the delivery of MSCs via a silk fibroin sponge scaffold, utilizing chitosan-based sponge scaffolds for MSC delivery could potentially lead to faster healing rates and increased collagen deposition. Moreover, significant reduction in ulcer size and improvement in wound closure time could also be noted.

MSC delivery by acellular bioscaffolds for DFU repair

Acellular bioscaffolds refer to biological substances that are derived from human or animal organs or tissues, which undergo decellularization techniques for the removal of immunogenic cellular components[159-162]. Acellular bioscaffolds have exhibited favorable results in diverse tissues and organs and have garnered noticeable interest in the domain of tissue engineering. Mechanical (freezing or force), chemical (acid or Triton), and enzymatic (trypsin or pepsin) methods are decellularization techniques that can be employed. While combining multiple techniques is often more effective than using a single method, it is crucial to choose the appropriate decellularization approach based on the distinctive features of each tissue type[163]. Acellular bioscaffolds are composed mainly of ECM and other extracellular macromolecules (e.g., collagen, elastin, fibronectin, laminin, and stromal cell proteins). They possess the unique characteristic of being nonimmunogenic 3D structures, distinguishing them from other synthetic scaffolds[161]. These characteristics are critical for the identification and development of implantable scaffolds for diabetic wounds. The utilization of acellular bioscaffolds has numerous benefits in treating diabetic wounds, involving the ability to replace damaged ECM with a variety of proteins (e.g., collagen, glycosaminoglycans, proteoglycans, and glycoproteins). Additionally, acellular bioscaffolds facilitate the infiltration of host cells and regulation of immune responses. They also promote angiogenesis and granulation tissue formation[164-165]. There are currently few commercially available acellular bioscaffolds for wound healing[166-167]. These scaffolds are manufactured differently and hence have differentmechanical properties and varying abilities to support skin regeneration[168]. Several studies have examined the delivery of MSCs by acellular bioscaffolds for the treatment of diabetic wounds. Shi et al.[169] developed a decellularized dermal matrix scaffold, called book-shaped decellularized dermal matrix (BDDM), closely resembling native dermal tissues in terms of histology, microstructure, and composition. This noncytotoxic scaffold exhibited low immunogenicity and supported the attachment and proliferation of ADMSCs. The researchers also synthesized a recombinant growth factor, CBD-bFGF, by fusing a collagen-binding domain (CBD) with bFGF, and tethered it to the collagen

fibers of the BDDM scaffold. This was resulted in the creation of a functional scaffold (CBD-bFF/BDDM), promoting endothelial cell inducibility more effectively. *In vitro* tests revealed that CBD-bFGF/BDDM scaffold can gradually release tethered bFGF and facilitate ADMSC interactions until endothelial differentiation is achieved. To evaluate the effectiveness of this scaffold, ADMSCs were cultured to create a cell sheet which was placed between placental growth factor, CBD-bFGF and BDDM before being transplanted into diabetic rats. Results from *in vivo* experiments showed that the implantation of ADMSC-loaded CBD-bFGF/BDDM scaffold promoted the formation of granulation tissue and angiogenesis. It also facilitated collagen deposition and remodeling. Zhang et al.[170] Zhang and colleagues developed a novel delivery system for exogenous cells using nanoparticles encapsulating IL-8 along with polylactic-co-glycolic acid (PLGA) loaded onto acellular matrix insulin-like growth factor 1. This efficient delivery medium, termed PLGA@IL-8/ADM, was found to promote significant proliferation and endothelial differentiation of the MSCs while increasing their survival rate. Moreover, PLGA@IL-8/ADM scaffold loaded with MSCs facilitated capillary construction, collagen deposition, and angiopoietin-1 wound healing in skin wounds of mice with STZ-induced diabetes, thereby demonstrating its effectiveness as a therapeutic intervention for diabetic wounds. These findings highlight the promise of the PLGA@IL-18/ADM scaffold as a novel delivery system for exogenous cells that can aid in tissue regeneration. Chu et al. conducted a study, in which they loaded mouse BMSCs onto a decellularized dermal matrix scaffold obtained from normal mouse skin and applied it at full-thickness cutaneous wound sites in diabetic mice. The use of MSC-ADM for treating these wounds resulted in a noteworthy increase in the percentage of wound closure, a boost in type I collagen fiber synthesis, and an acceleration of both angiogenesis and re-epithelization[171-173]. Moreover, a cell delivery platform comprised of acellular dermal matrix and reduced graphene oxide has high stability and promisingmechanical properties. Upon delivery of murine BMSCs to woundsindiabetic mice, this acellular bioscaffold provides a favorable milieu for BMSC adhesion and proliferation, promotes angiogenesis and collagen deposition, and accelerates wound

healing[174-175]. A study using acellular dermal matrix to deliver human umbilical cord MSCs showed that MSC proliferation and differentiation were regulated by activation of Wnt signaling, which ultimately promoted wound healing in diabetic rats[176].

Matrix formed by the removal ofcellsfrom dermal tissues should be used to deliver MSCs to wounds, thereby resolving immune rejection of allografts. Research priorities related tothe use of decellularized vascular bioscaffolds for MSC delivery and wound repair include cell growth promotion, vascularization, and appendage regeneration, which may represent the future of DFU treatment.

Acellular bioscaffolds have demonstrated their efficacy as a successful delivery platform for MSCs, facilitating a supportive environment for cell growth and driving tissue regeneration at the site of the ulcer. The use of acellular bioscaffolds can modulate the immune response and reduce inflammation at the wound site, aiding in the healing process. MSCs delivered by acellular bioscaffolds can improve angiogenesis and blood vessel formation, increasing blood flow to the ulcerated area and promoting healing. The controlled release of MSCs from acellular bioscaffolds can provide sustained therapeutic effects over time, reducing the need for frequent treatments. The use of acellular bioscaffolds may improve patient compliance and reduce the risk of infection compared with other methods of MSC delivery. Overall, MSCs can promote wound healing by secreting cytokines and growth factors, reducing inflammation, and stimulating angiogenesis. However, delivering MSCs directly to the siteofDFUwoundscan be challenging due to low survival rates and poor engraftment. Using an acellular bioscaffold as a delivery vehicle could potentially improve the viability and functionality of transplanted MSCs and enhance their therapeutic effects for DFUs. The bioscaffold provides a 3D microenvironment similar to the natural ECM, allowing for cell attachment and migration, promoting angiogenesis, and increasing nutrient and oxygen availability.

CONCLUSION

In conclusion, the increasing incidence of nontraumatic amputation and the poor therapeutic effect of currently available treatments make DFU one of the most important clinical challenges[177]. MSCs are widely utilized in the treatment of DFU, however, their efficacy needs to be improved. The application of different MSCs-based drug delivery systemsfor DFU and the relevant mechanisms were discussed (Table 1). Several preclinical investigations have exhibited impressive results, indicating that diverse MSCs-based drug delivery mechanisms can expedite wound healing and stimulate skin regeneration in DFU. However, there are still limited clinical data regarding the utilization of MSCs-based drug delivery systems for treating DFU. There is no consistent correlation between the results obtained in animal and human models. The safety, efficacy, and cost of different MSCs-based drug delivery systems should be deeply investigatedin the future research. An interdisciplinary approach is required to develop cells-based drug delivery systemsfor the clinical treatment of DFU.

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